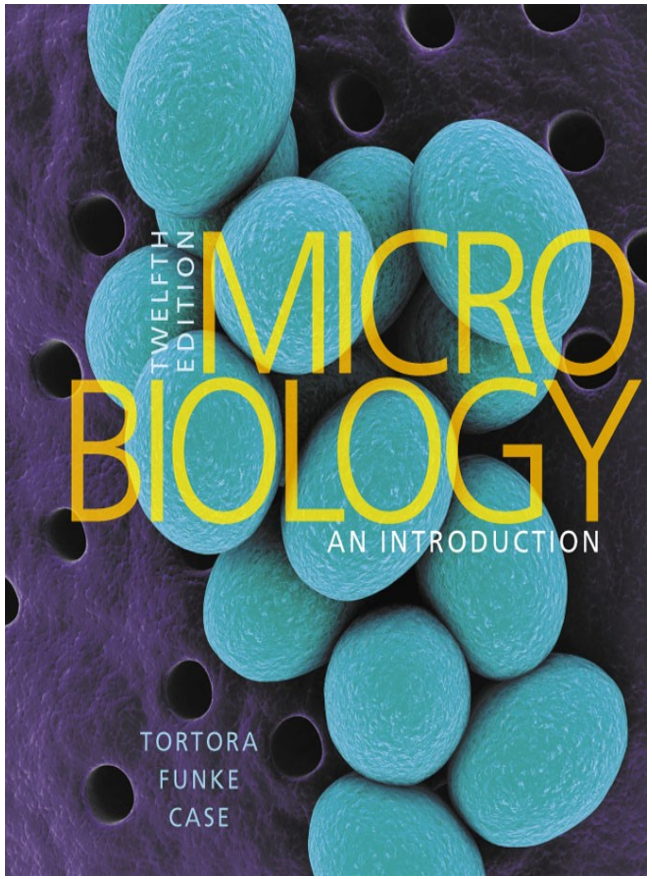
Microbiology an Introduction

Twelfth Edition



Chapter 3

Observing Microorganisms through a Microscope

Pearson

Helicobacter Pylori Bacteria

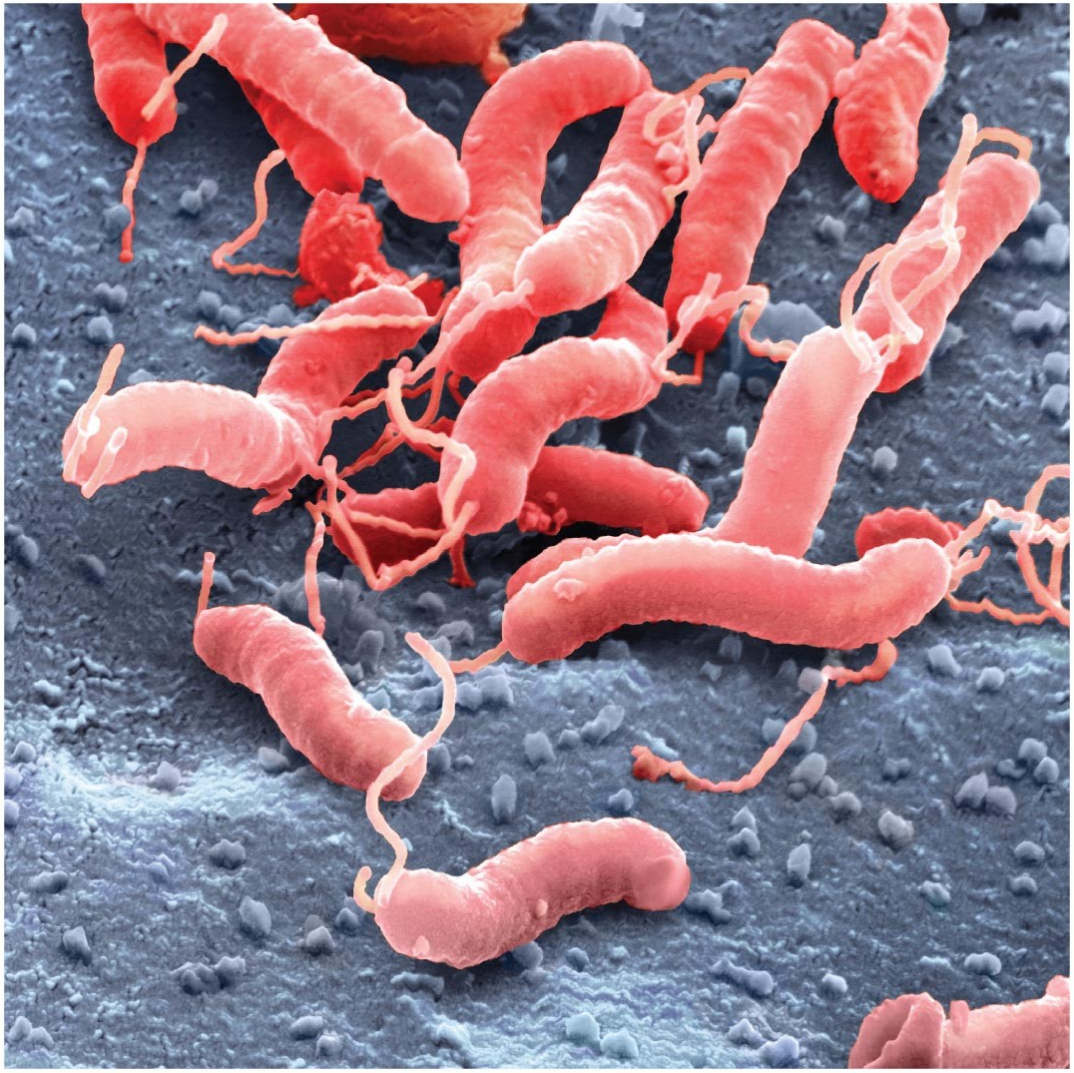
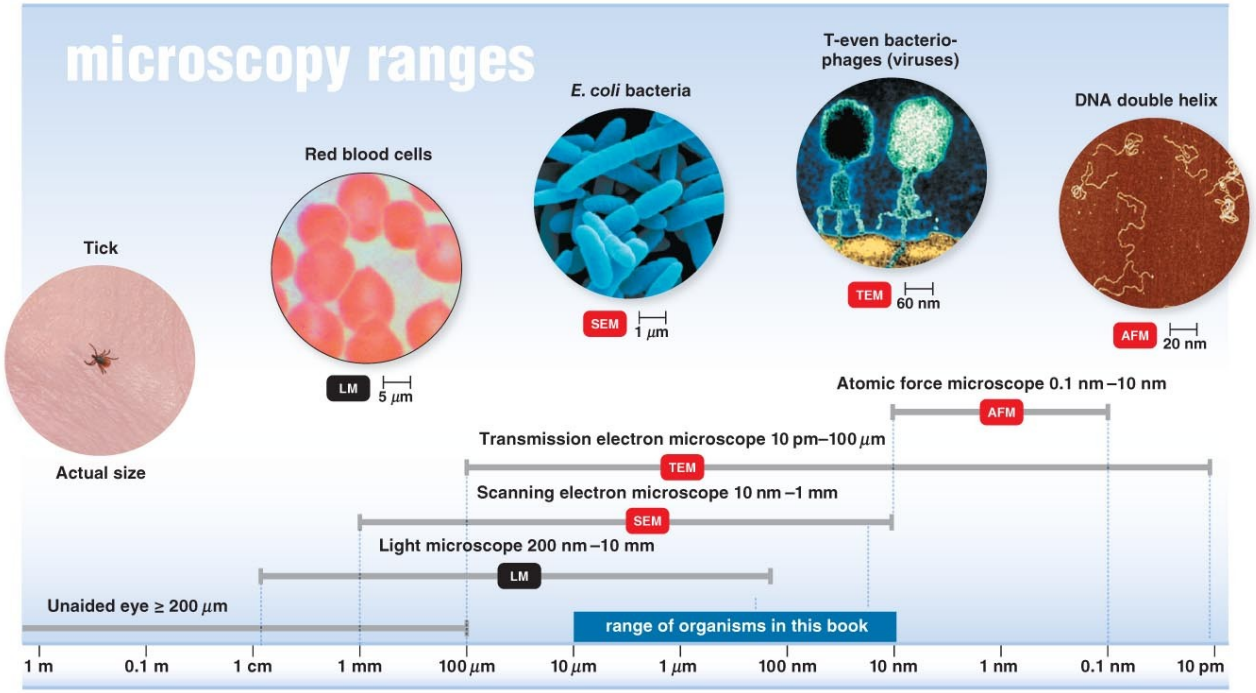


Figure 3.2 Microscopes and Magnification



KEY CONCEPTS

Microscopes are used to magnify small objects.

Because different microscopes have different resolution ranges, the size of a specimen determines which microscopes can be used to view the specimen effectively.

Most micrographs shown in this textbook (like the ones below) have size bars and symbols to help you identify the actual size of the specimen and the type of microscope used for that image.

A red icon indicates that a micrograph has been artificially colorized.

Resolution increases with decreasing wavelength.

micro tip

If a bacterium is 1 micrometer long and your index finger is 6.5 cm long, how many of the bacteria can you place end-to-end on your finger? Answer: 32,500.

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Units of Measurement (1 of 2)

Learning Objective

3-1 List the units used to measure microorganisms.

Units of Measurement (2 of 2)

- Microorganisms are measured in **micrometers** (μm) and **nanometers** (nm)
- $1\,\mu m = 10^{-6}\ m = 10^{-3}\ mm$
- $1\,nm = 10^{-9}\ m = 10^{-6}\ mm$
- $1000\,nm = 1\,\mu m$
- $0.001\,\mu m = 1\,nm$

Check Your Understanding-1

Check Your Understanding

- ✓ How many nanometers is 10 μm?
  3-1

Microscopy: The Instruments (1 of 2)

Learning Objectives

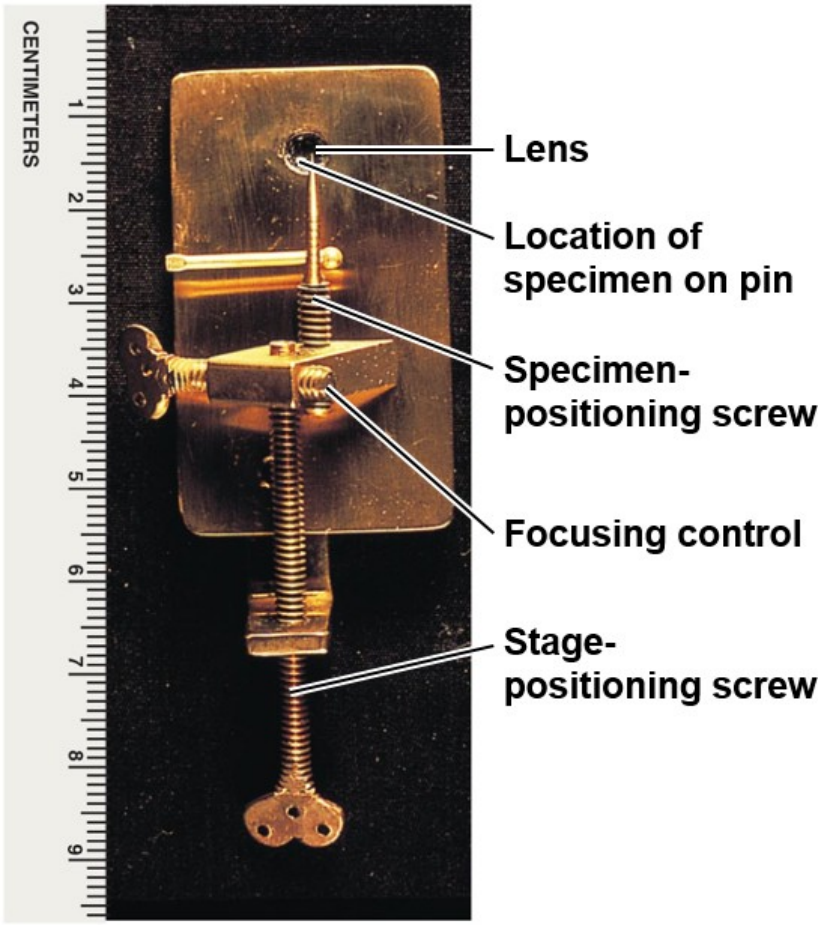
3-2 Diagram the path of light through a compound microscope.

3-3 Define **total magnification** and **resolution**.

Microscopy: The Instruments (2 of 2)

- A simple microscope has only one lens

Figure 1.2b Anton van Leeuwenhoek's Microscopic Observations

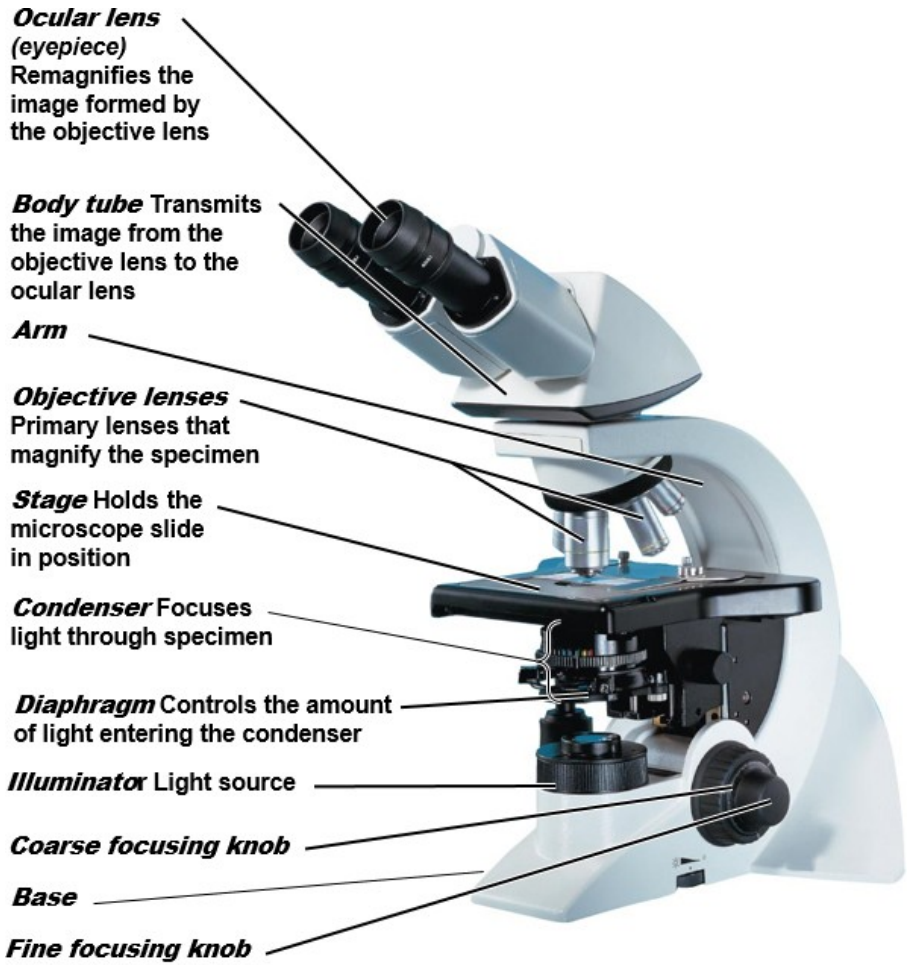


(b) Microscope replica

Light Microscopy (1 of 2)

- Any kind of microscope that uses visible light to observe specimens
- Types of **light microscopy**
  - Compound light microscopy
  - Darkfield microscopy
  - Phase-contrast microscopy
  - Differential interference contrast (DIC) microscopy
  - Fluorescence microscopy
  - Confocal microscopy

Figure 3.1a The Compound Light Microscope



(a) Principal parts and functions

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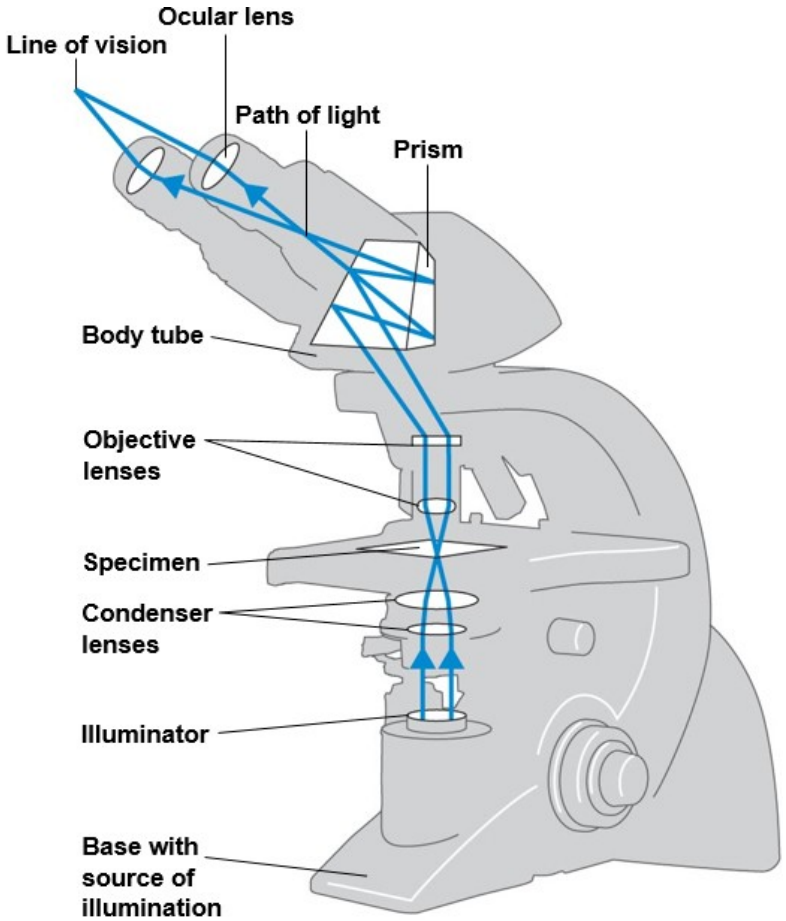
Compound Light Microscopy (1 of 4)

- In a **compound microscope,** the image from the objective lens is magnified again by the **ocular lens**
- **Total magnification** = objective lens × ocular lens

Figure 3.1b The Compound Light Microscope



(b) The path of light (bottom to top)

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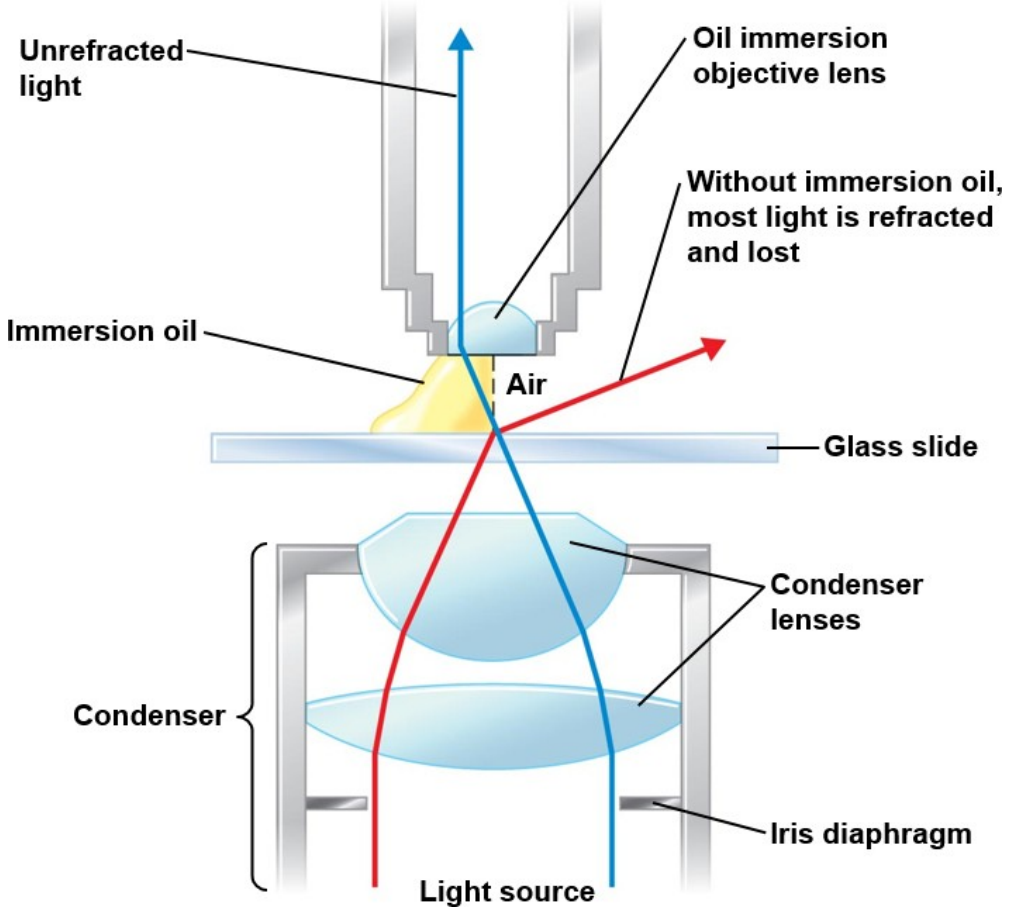
Compound Light Microscopy (2 of 4)

- **Resolution** is the ability of the lenses to distinguish two points
- A microscope with a resolving power of 0.4 nm can distinguish between two points at least 0.4 nm apart
- Shorter wavelengths of light provide greater resolution

Compound Light Microscopy (3 of 4)

- The **refractive index** is a measure of the light-bending ability of a medium
- Light may refract after passing through a specimen to an extent that it does not pass through the objective lens
- Immersion oil is used to keep light from refracting

Figure 3.3 Refraction in the Compound Microscope Using an Oil Immersion Objective Lens

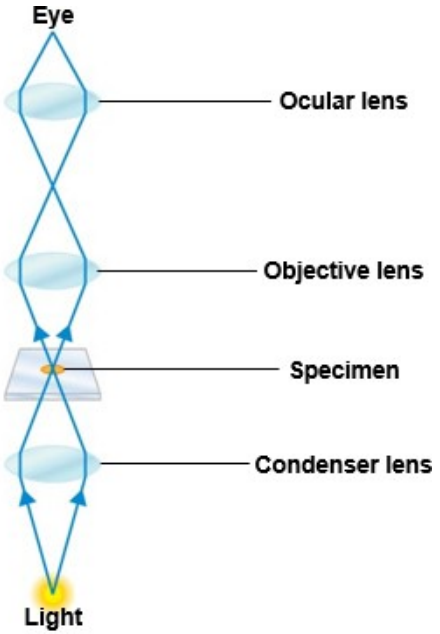


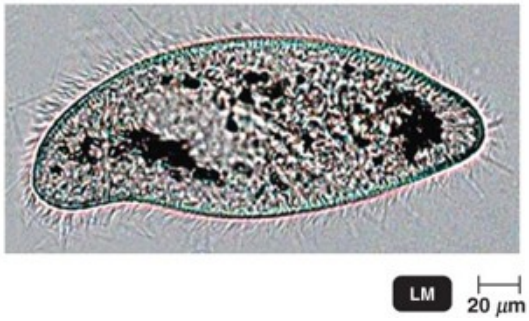
Compound Light Microscopy (4 of 4)

- **Brightfield illumination**
  - Dark objects are visible against a bright background
  - Light reflected off the specimen does not enter the objective lens

Figure 3.4a Brightfield, Darkfield, and Phase-Contrast Microscopy

(a). Brightfield. (Top) The path of light in brightfield microscopy, the type of illumination produced by regular compound light microscopes. (Bottom) Brightfield illumination shows internal structures and the outline of the transparent pellicle (external covering).





Light Microscopy (2 of 2)

PLAY **Animation: Light Microscopy**

Check Your Understanding-2

Check Your Understanding

- ✓ Through what lenses does light pass in a compound microscope?
  3-2
- ✓ What does it mean when a microscope has a resolution of 0.2 nm?
  3-3

Microscopy: The Instruments

Learning Objectives

3-4 Identify a use for darkfield, phase-contrast, differential interference contrast, fluorescence, confocal, two-photon, and scanning acoustic microscopy, and compare each with brightfield illumination.

3-5 Explain how electron microscopy differs from light microscopy.

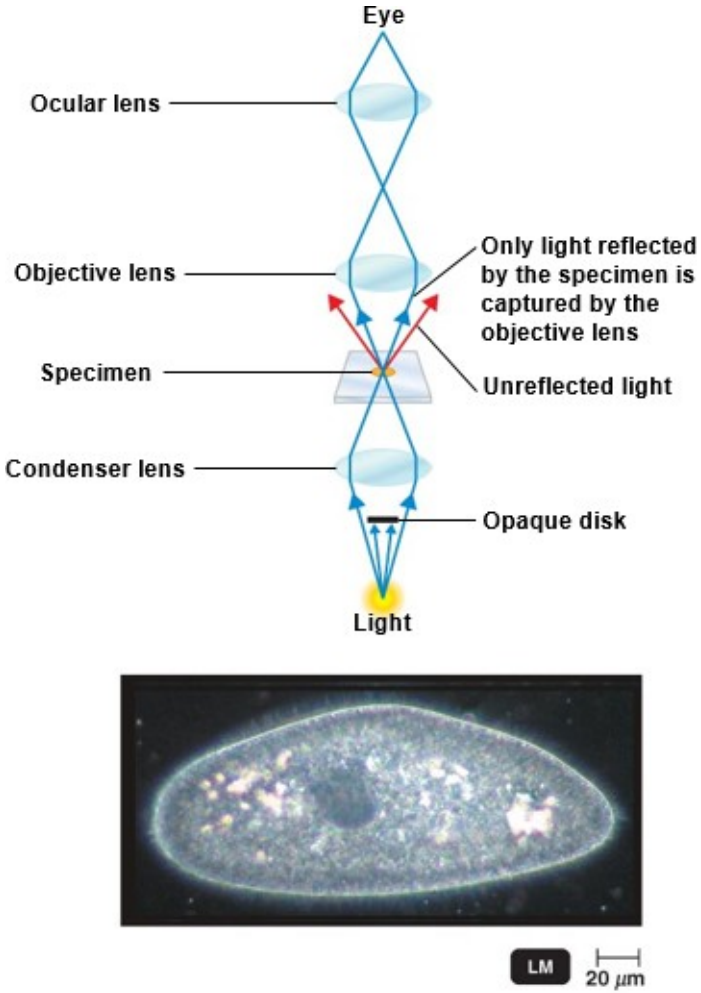
3-6 Identify uses for the transmission electron microscope (TEM), scanning electron microscope (SEM), and scanned-probe microscopes.

Darkfield Microscopy

- Light objects are visible against a dark background
- Opaque disk placed in condenser
- Only light reflected off the specimen enters the objective lens

Figure 3.4b Brightfield, Darkfield, and Phase-Contrast Microscopy

(b) Darkfield. (Top) The darkfield microscope uses a special condenser with an opaque disk that eliminates all light in the center of the beam. The only light that reaches the specimen comes in at an angle; thus, only light reflected by the specimen (blue lines) reaches the objective lens. (Bottom) Against the black background seen with darkfield microscopy, edges of the cell are bright, some internal structures seem to sparkle, and the pellicle is almost visible.

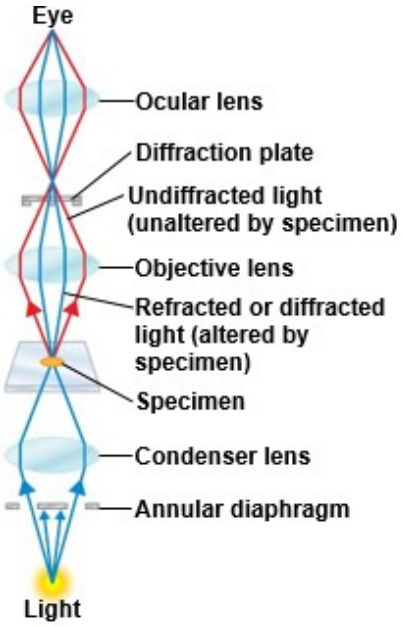


Phase-Contrast Microscopy

- Allows examination of living organisms and internal cell structures
- Brings together two sets of light rays, direct rays, and diffracted rays to form an image

Figure 3.4c Brightfield, Darkfield, and Phase-Contrast Microscopy

(c). Phase-contrast. (Top) In phase-contrast microscopy, the specimen is illuminated by light passing through an annular (ring-shaped) diaphragm. Direct light rays (unaltered by the specimen) travel a different path from light rays that are reflected or diffracted as they pass through the specimen. These two sets of rays are combined at the eye. Reflected or diffracted light rays are indicated in blue; direct rays are red. (Bottom) Phase-contrast microscopy shows greater differentiation of internal structures and clearly shows the pellicle



Differential Interference Contrast (DIC) Microscopy

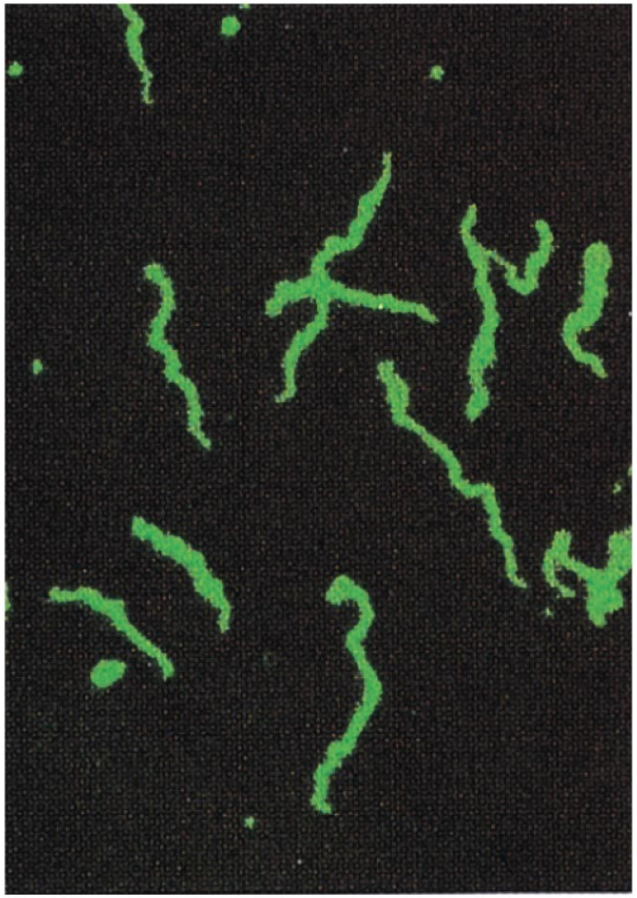
- Similar to phase-contrast
- Uses two light beams and prisms to split light beams, giving more contrast and color to the specimen

Figure 3.5 Differential Interference Contrast (DIC) Microscopy

Fluorescence Microscopy

- Uses UV (short wavelength) light
- Fluorescent substances absorb UV light and emit longer wavelength (visible) light
- Cells may be stained with fluorescent dyes (fluorochromes) if they do not naturally fluoresce

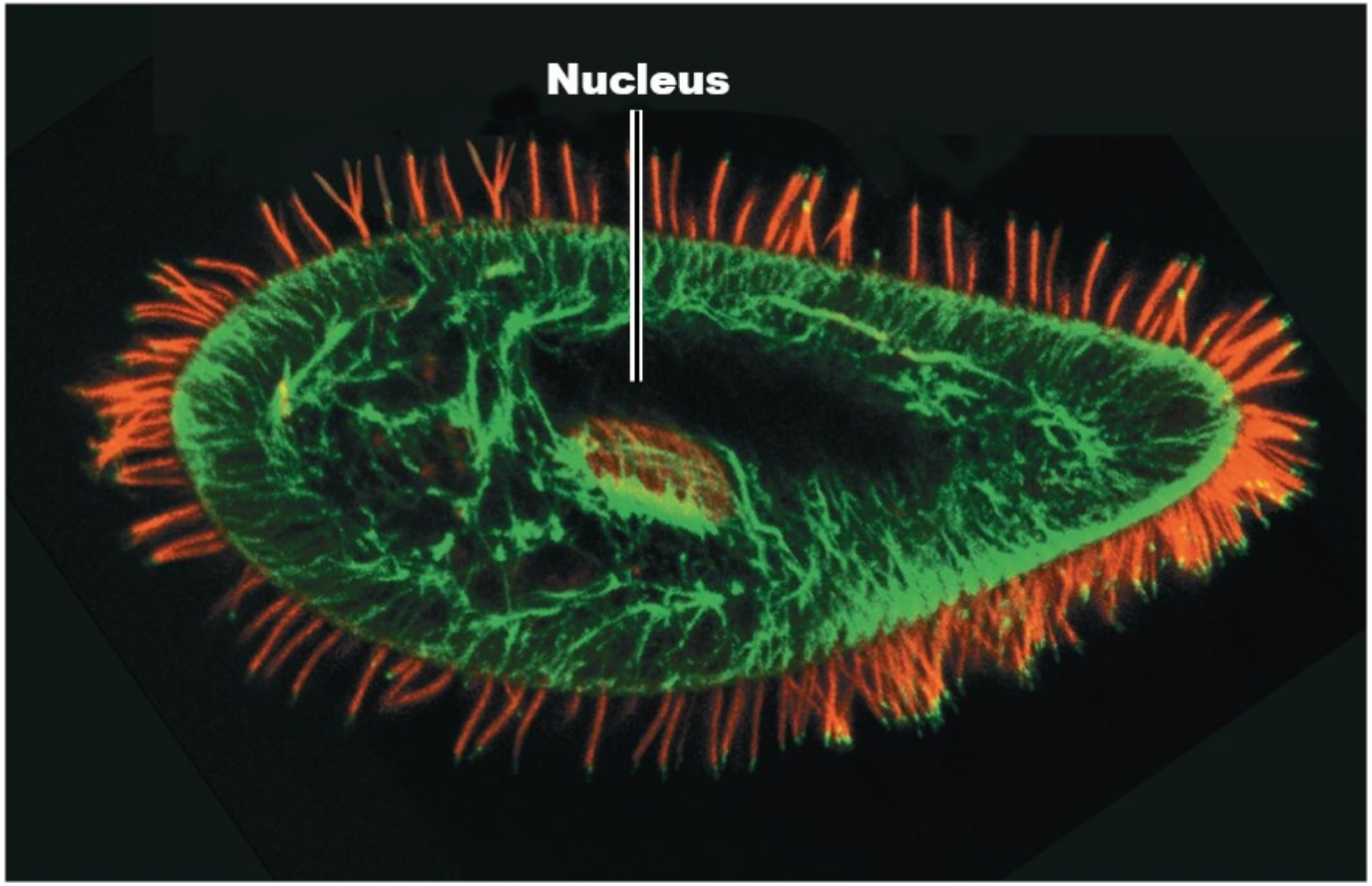
Figure 3.6b The Principle of Immunofluorescence



Confocal Microscopy

- Cells are stained with fluorochrome dyes
- Short-wavelength (blue) light is used to excite a single plane of a specimen
- Each plane in a specimen is illuminated and a three-dimensional image is constructed with a computer

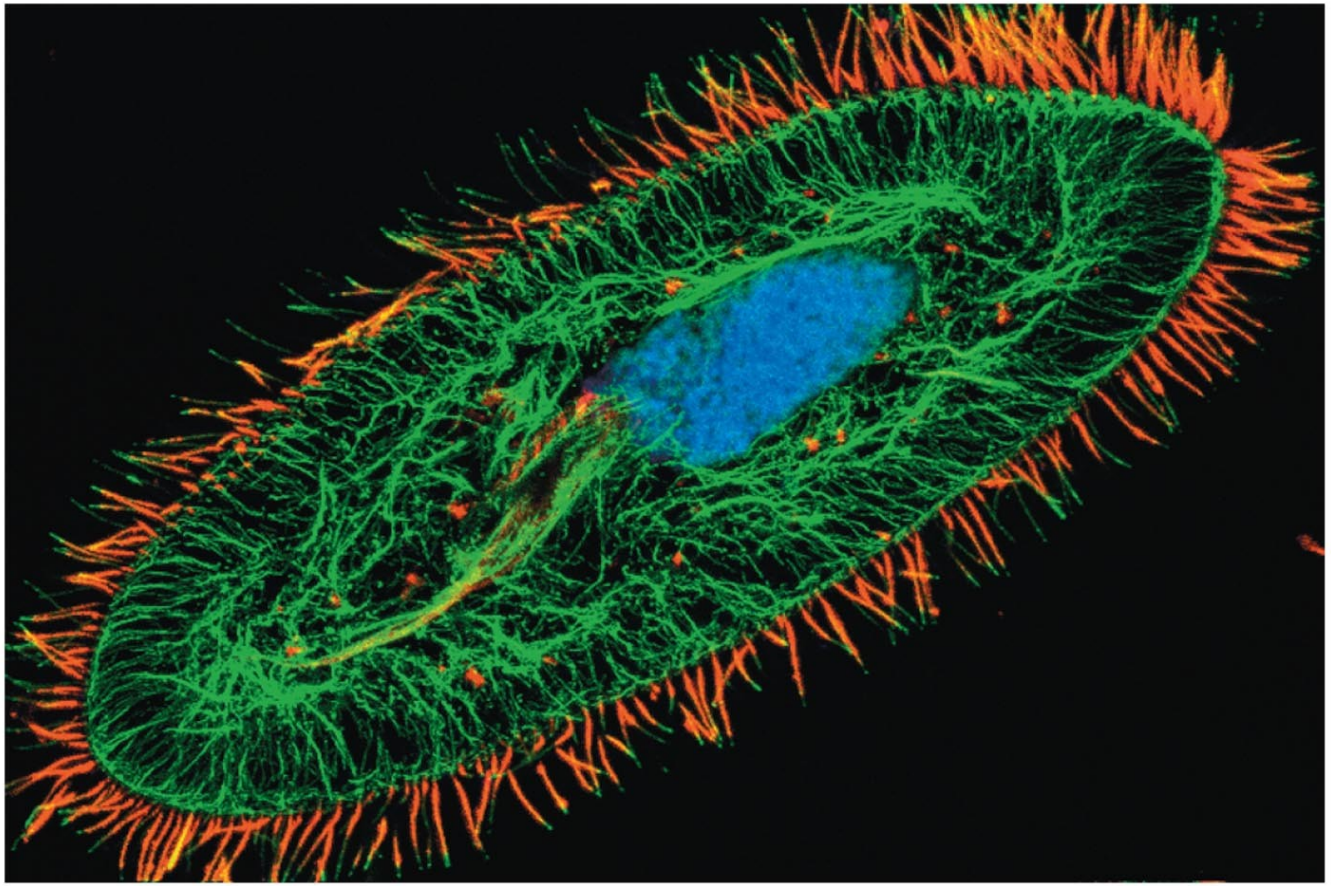
Figure 3.7 Confocal Microscopy



Two-Photon Microscopy

- Cells are stained with fluorochrome dyes
- Two photons of long-wavelength (red) light are used to excite the dyes
- Can study living cells up to 1 mm deep

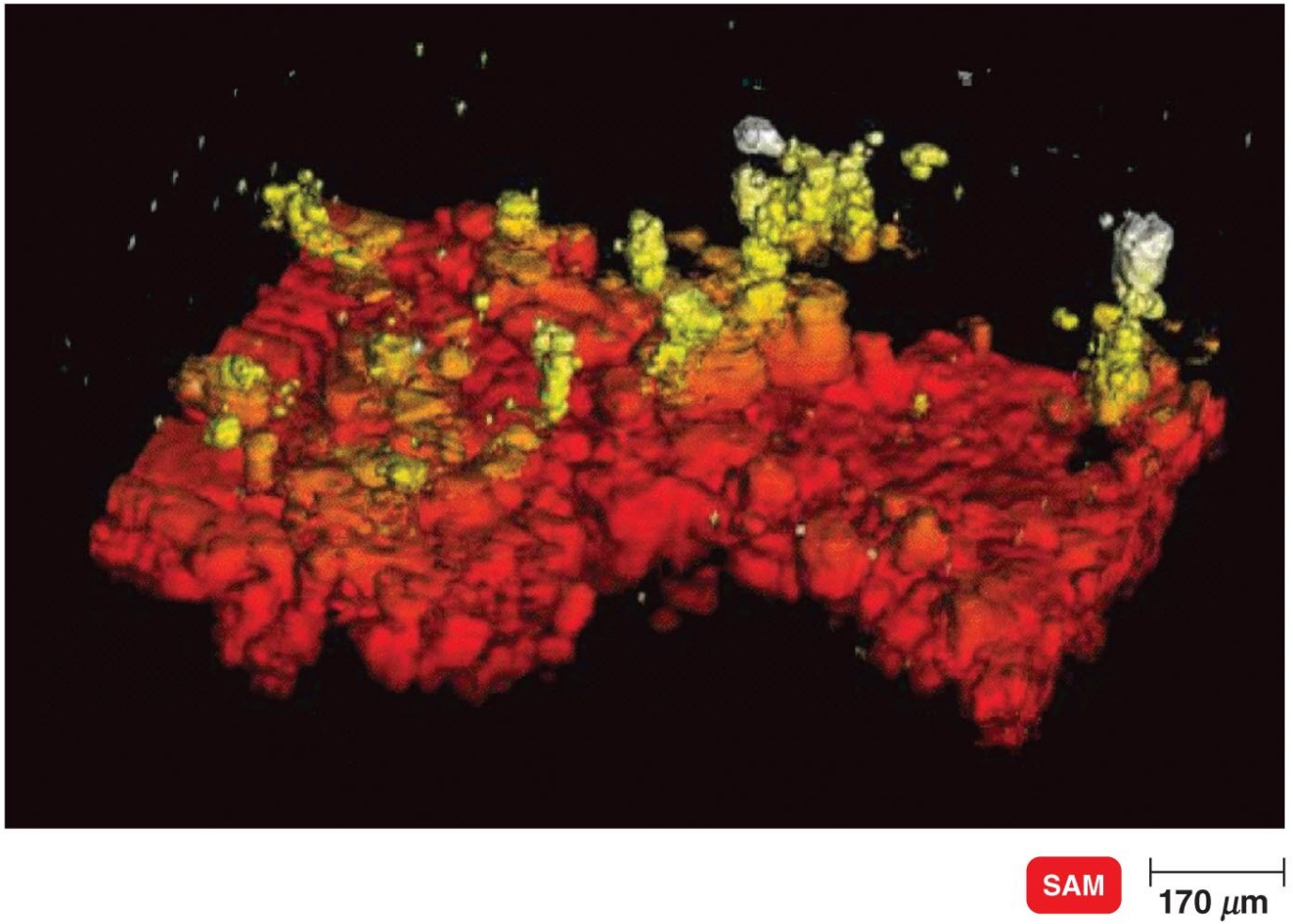
Figure 3.8 Two-Photon Microscopy (TPM)



Scanning Acoustic Microscopy

- Measures sound waves that are reflected back from a specimen
- Used to study cells attached to surfaces
- Resolution of 1 μm

Figure 3.9 Scanning Acoustic Microscopy (SAM) of a Bacterial Biofilm on Glass



Check Your Understanding-3

Check Your Understanding

- ✓ How are brightfield, darkfield, phase-contrast, and fluorescence microscopy similar?
  3-4

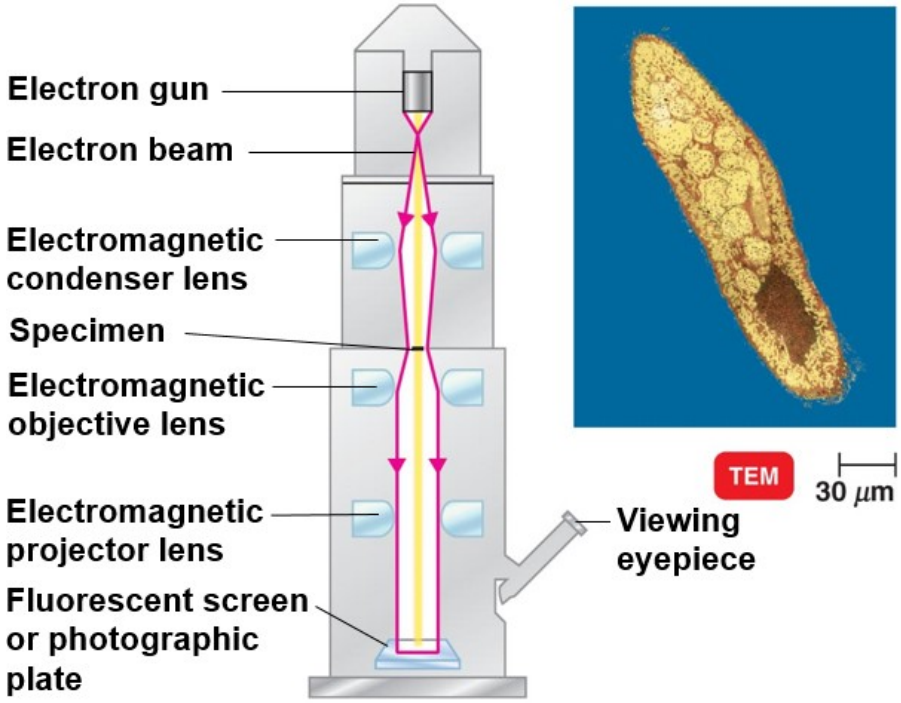
Electron Microscopy (1 of 2)

- Uses electrons instead of light
- The shorter wavelength of electrons gives greater resolution
- Used for images too small to be seen with light microscopes, such as viruses

Transmission Electron Microscopy (1 of 2)

- A beam of electrons passes through ultrathin sections of a specimen, then through an electromagnetic lens, then focused on a projector lens
- Specimens may be stained with heavy-metal salts for contrast

Figure 3.10a Transmission and Scanning Electron Microscopy



(a). Transmission. (Left) In a transmission electron microscope, electrons pass through the specimen and are scattered. Magnetic lenses focus the image onto a fluorescent screen or photographic plate. (Right) This colorized transmission electron micrograph (TEM) shows a thin slice of **Paramecium**. In this type of microscopy, the internal structures present in the slice can be seen.

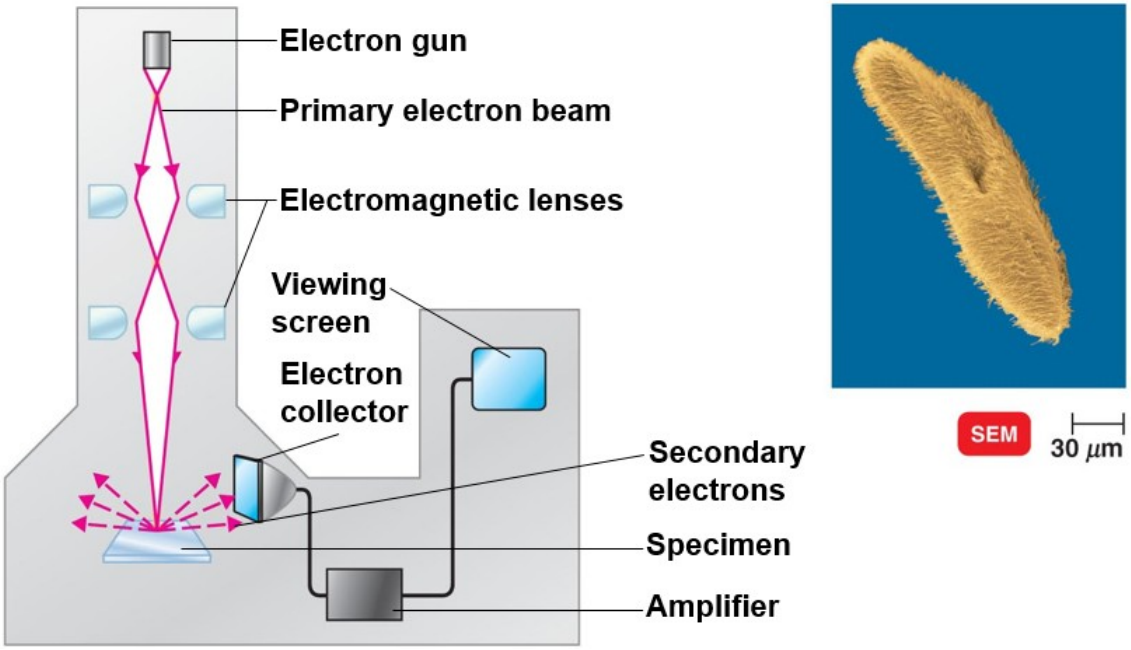
Transmission Electron Microscopy (2 of 2)

- Magnifies objects 10,000 to 100,000×; resolution of 10 pm

Scanning Electron Microscopy (1 of 2)

- An electron gun produces a beam of electrons that scans the surface of an entire specimen
- Secondary electrons emitted from the specimen produce a three-dimensional image

Figure 3.10b Transmission and Scanning Electron Microscopy



(b) Scanning. (Left) In a scanning electron microscope, primary electrons sweep across the specimen and knock electrons from its surface. These secondary electrons are picked up by a collector, amplified, and transmitted onto a viewing screen or photographic plate. (Right) In this colorized scanning electron micrograph (SEM), the surface structures of **Paramecium** can be seen. Note the three-dimensional appearance of this cell, in contrast to the two-dimensional appearance of the transmission electron micrograph in part (a).

Scanning Electron Microscopy (2 of 2)

- Magnifies objects 1000 to 10,000×; resolution of 10 nm

Electron Microscopy (2 of 2)

PLAY **Animation: Electron Microscopy**

Check Your Understanding-4

Check Your Understanding

- ✓ Why do electron microscopes have greater resolution than light microscopes?
  3-5

Scanning Tunneling Microscopy

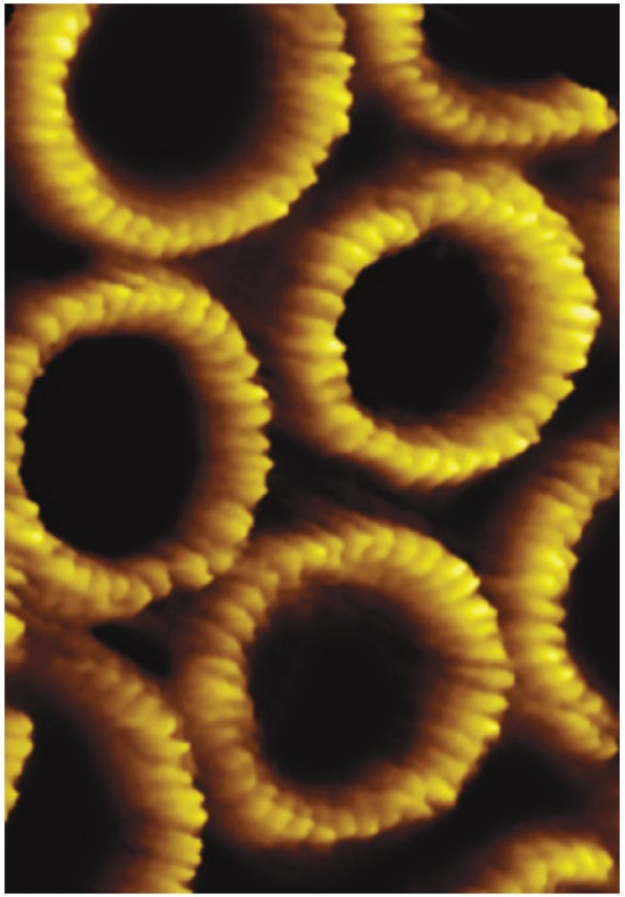
- Uses a tungsten probe to scan a specimen and reveal details of its surface
- Resolution of 1/100 of an atom

Figure 3.11a Scanned-Probe Microscopy

Atomic Force Microscopy

- Uses a metal-and-diamond probe placed onto a specimen
- Produces three-dimensional images

Figure 3.11b Scanned-Probe Microscopy



Check Your Understanding-5

Check Your Understanding

- ✓ For what is TEM used? SEM? Scanned-probe microscopy?
  3-6

Preparation of Specimens for Light Microscopy

Learning Objectives

3-7 Differentiate an acidic dye from a basic dye.

3-8 Explain the purpose of simple staining.

3-9 List Gram stain steps, and describe the appearance of gram-positive and gram-negative cells after each step.

3-10 Compare and contrast the Gram stain and the acid-fast stain.

3-11 Explain why each of the following is used: capsule stain, endospore stain, flagella stain.

Preparing Smears for Staining
(1 of 3)

- **Staining:** coloring microorganisms with a dye that emphasizes certain structures
- **Smear:** a thin film of a material containing microorganisms spread over a slide
- Microorganisms are **fixed** (attached) to the slide, which kills the microorganisms

Preparing Smears for Staining
(2 of 3)

- Live and/or unstained specimens have little contrast with the surrounding medium. Live specimens are used to study cell behavior.

Microscopy and Staining: Overview

Animation: Microscopy and Staining: Overview

Preparing Smears for Staining
(3 of 3)

- Stains consist of a positive and negative ion, one of which is colored (**chromophore**)
- In a **basic dye,** the chromophore is a cation
- In an **acidic dye,** the chromophore is an anion
- Staining the background instead of the cell is called **negative staining**

Simple Stains

- **Simple stain:** use of a single basic dye
- Highlights the entire microorganism to visualize cell shapes and structures
- A **mordant** may be used to hold the stain or coat the specimen to enlarge it

Staining

Animation: Staining

Check Your Understanding-6

Check Your Understanding

- ✓ Why doesn't a negative stain color a cell?
  3-7
- ✓ Why is fixing necessary for most staining procedures?
  3-8

Differential Stains

- Used to distinguish between bacteria
  - **Gram stain**
  - **Acid-fast stain**

Gram Stain

- Classifies bacteria into **gram-positive** or **gram-negative**
  - Gram-positive bacteria have thick peptidoglycan cell walls
  - Gram-negative bacteria have thin peptidoglycan cell walls and a layer of lipopolysaccharides

Figure 3.12a Gram Staining

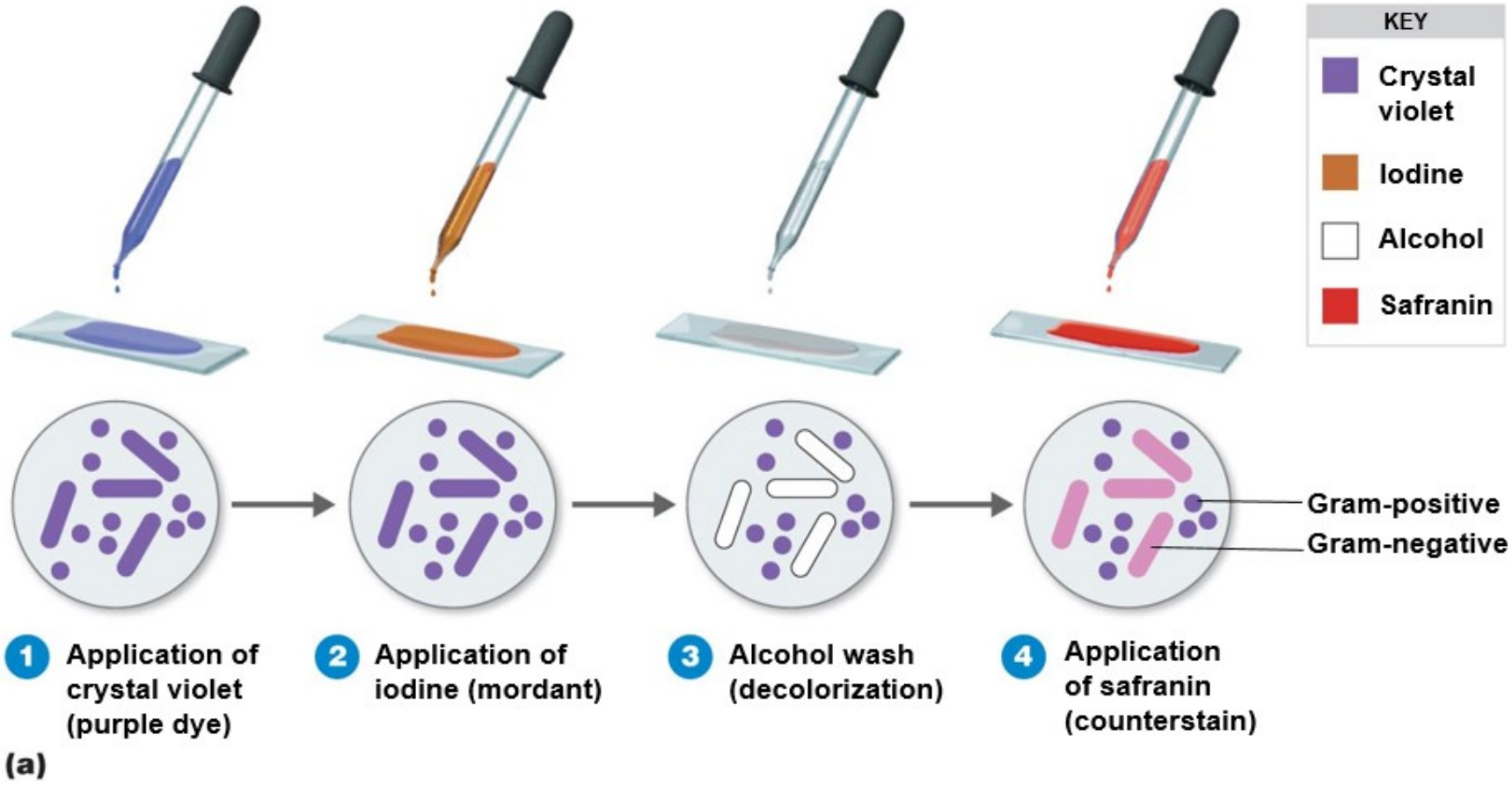
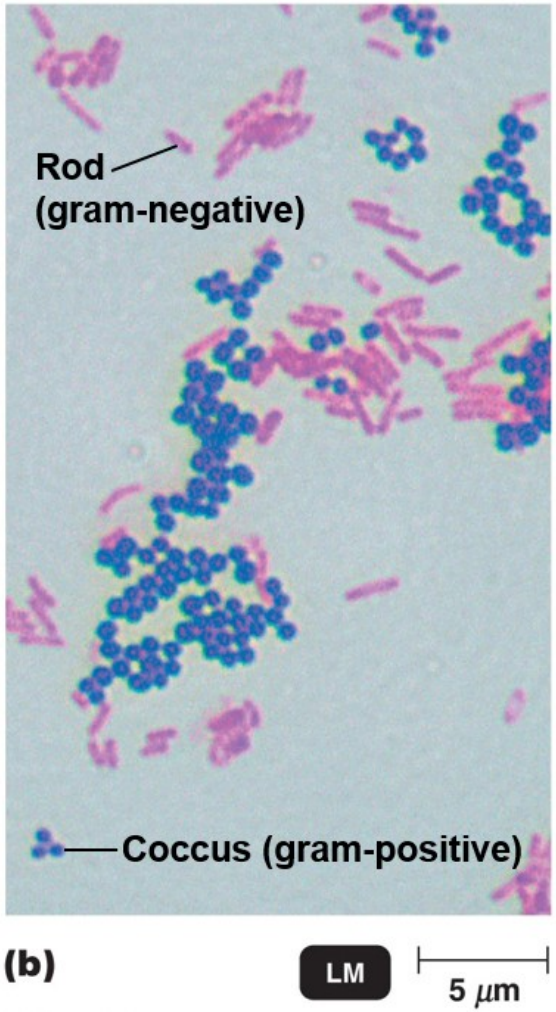


Figure 3.12b Gram Staining



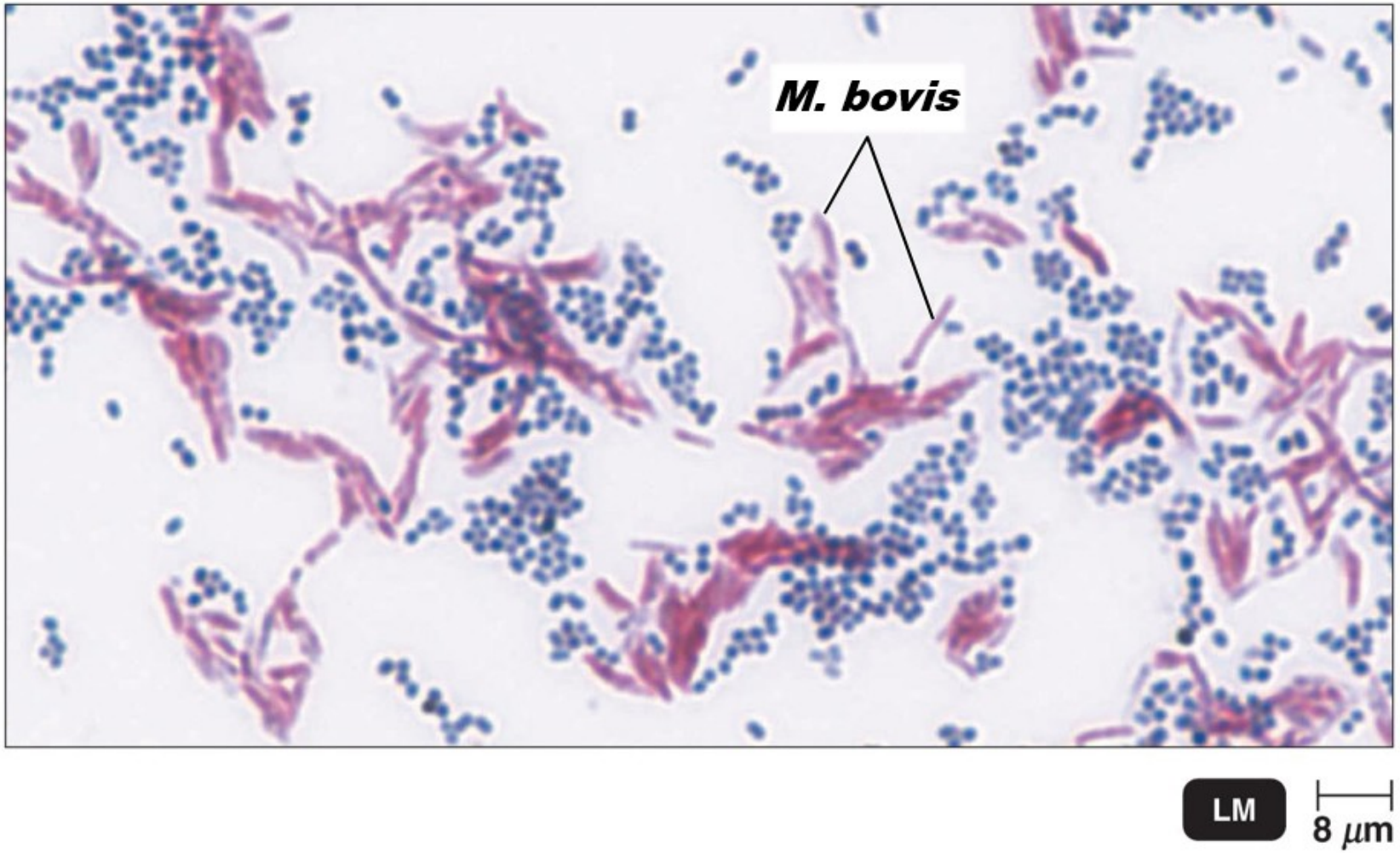
Acid-Fast Stain (1 of 2)

- Binds only to bacteria that have a waxy material in their cell walls, which is not decolorized by acid-alcohol
- Used for the identification of
  - **Mycobacterium**
  - **Nocardia**

Acid-Fast Stain (2 of 2)

	Color of Acid-Fast	Color of Non–Acid-Fast
Primary Stain: Carbolfuchsin	Red	Red
Decolorizing Agent: Acid-alcohol	Red	Colorless
Counterstain: Methylene Blue	Red	Blue

Figure 3.13 Acid-Fast Bacteria



Check Your Understanding-7

Check Your Understanding

- ✓ Why is the Gram stain so useful?
  3-9
- ✓ Which stain would be used to identify microbes in the genera **Mycobacterium** and **Nocardia**?
  3-10

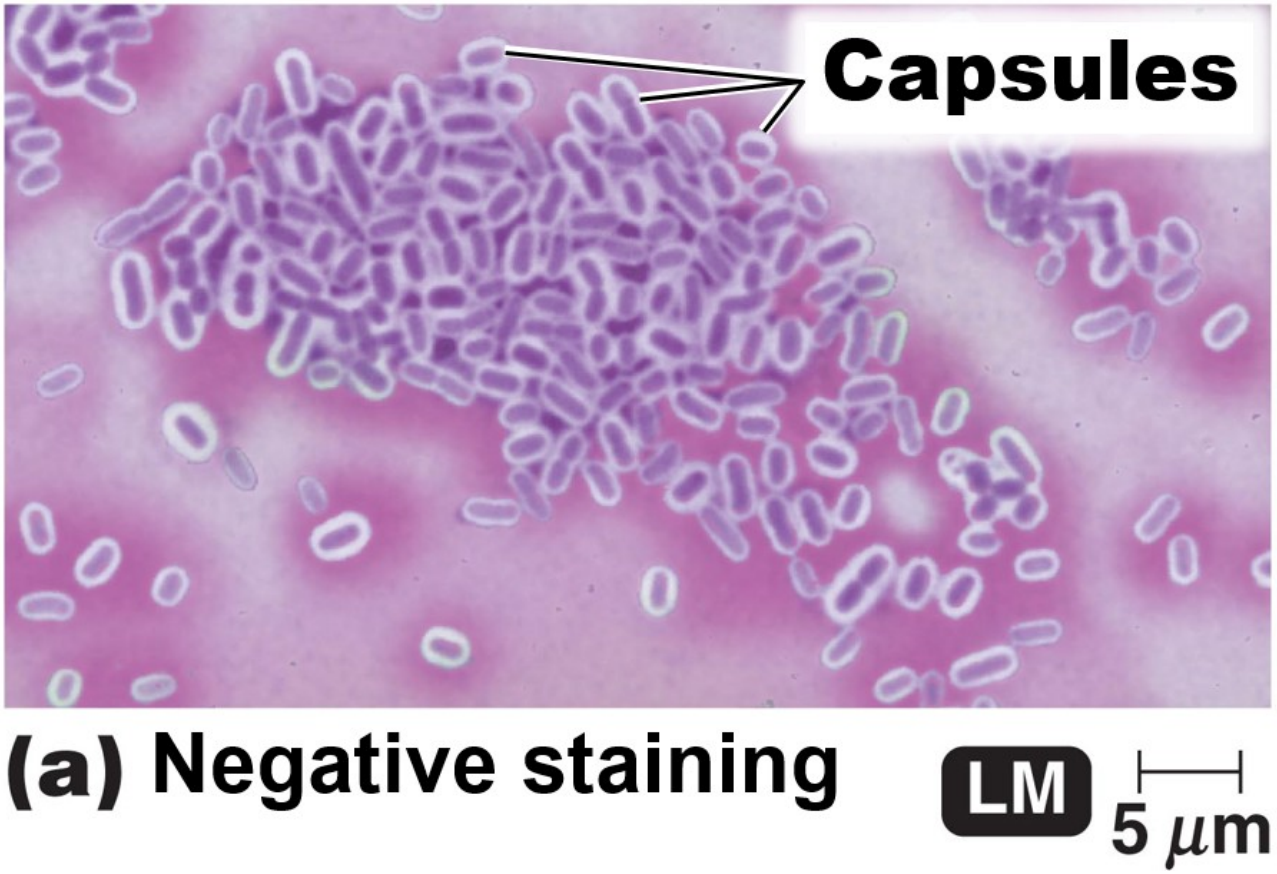
Special Stains

- Used to distinguish parts of microorganisms
  - Capsule stain
  - Endospore stain
  - Flagella stain

Negative Staining for Capsules

- **Capsules** are a gelatinous covering that do not accept most dyes
- Suspension of India ink or nigrosin contrasts the background with the capsule, which appears as a halo around the cell

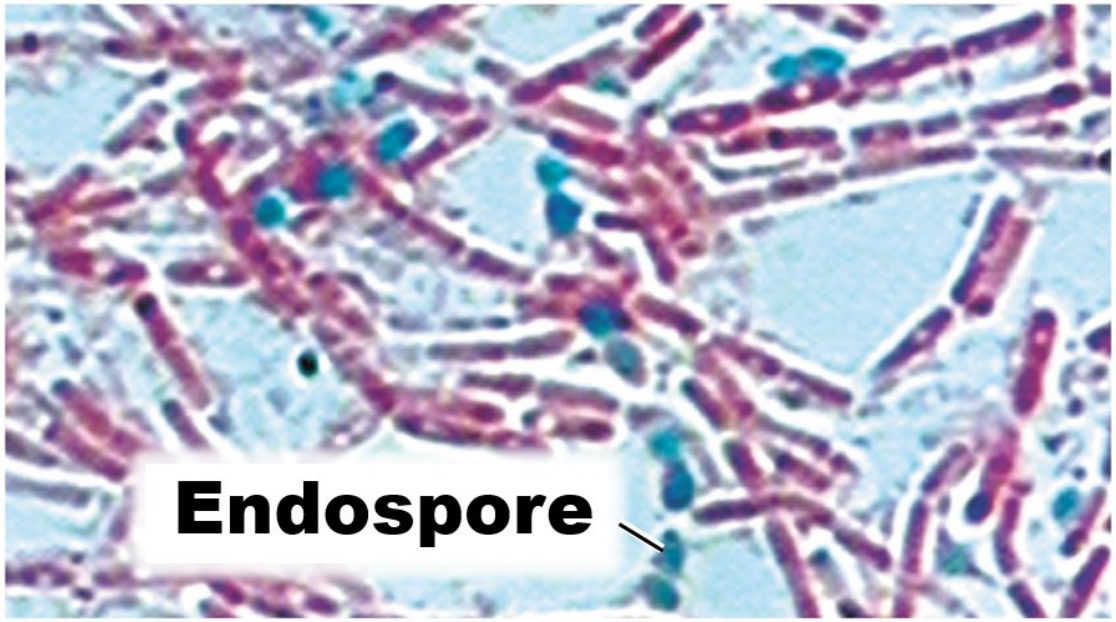
Figure 3.14a Special Staining



Endospore Staining

- **Endospores** are resistant, dormant structures inside some cells that cannot be stained by ordinary methods
- Primary stain: malachite green, usually with heat
- Decolorize cells: water
- Counterstain: safranin
- Spores appear green within red or pink cells

Figure 3.14b Special Staining

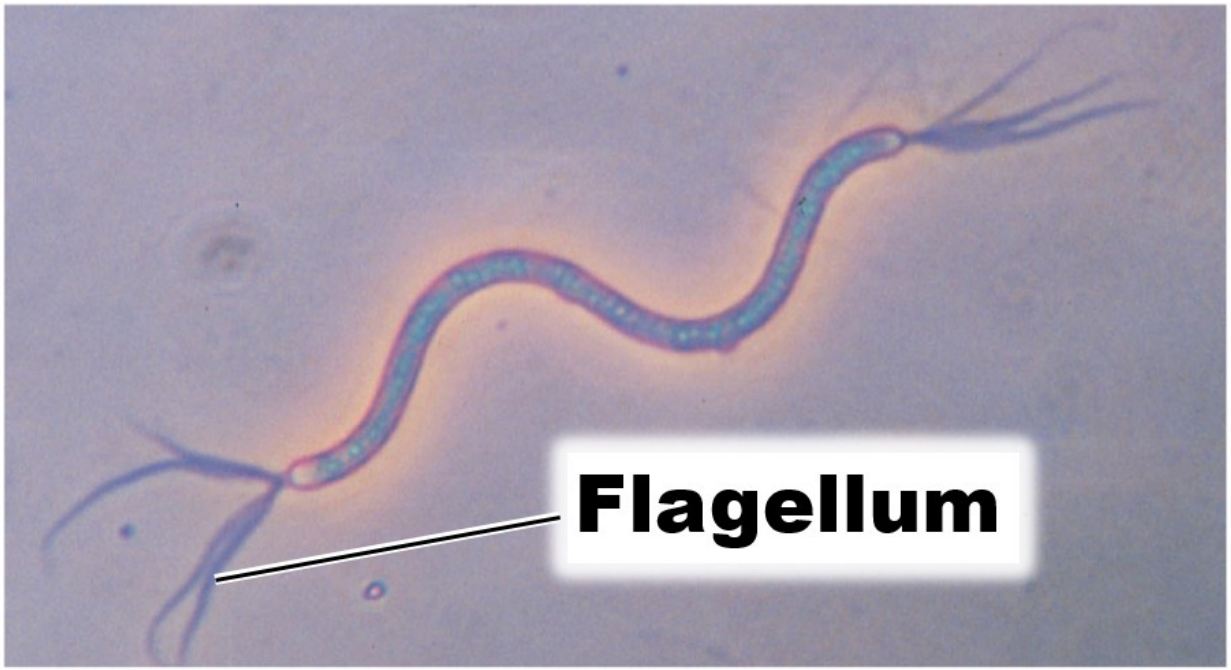


(b) Endospore staining

Flagella Staining

- **Flagella** are structures of locomotion
- Uses a mordant and carbolfuchsin

Figure 3.14c Special Staining



Check Your Understanding-8

Check Your Understanding

- ✓ How do unstained endospores appear? Stained endospores?
  3-11